

Amendments to the Specification:

Please insert the following new sections on page 1, after the Title, as shown below:

CROSS-REFERENCED TO RELATED APPLICATION

This application is the U.S. national phase of PCT Appln. No. PCT/GB2004/04907 filed November 19, 2004 which claims priority to Great Britain application GB 0327179.8 filed November 21, 2003.

STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT

Not applicable.

THE NAMES OF THE PARTIES TO A JOINT RESEARCH AGREEMENT

Not applicable.

INCORPORATION-BY-REFERENCE OF MATERIAL
SUBMITTED ON A COMPACT DISC

Not applicable.

SUMMARY

The present invention relates to a method for obtaining a recombinant glucose binding protein, in particular the lectin Concanavalin A (Con A). The method specifically utilizes a buffer in which impurities, such as glycogen and other substances are soluble, but in

which the protein remains soluble. The use of such buffers, and the purified proteins are also described.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 depicts dextran affinity chromatography elution profile for recombinant mature Con A refolded from the bacterial cell fraction insoluble in MOPS-metals buffer;

FIGURE 2 depicts UV spectral wavelength scan after affinity chromatography of recombinant mature Con A (refolded from the bacterial cell fraction insoluble in MOPS-metals buffer);

FIGURE 3 depicts dextran affinity chromatography elution profile for recombinant mature Con A refolded from the fraction insoluble in MOPS-metals buffer obtained from glycogen-deficient mutant bacterial cells;

FIGURE 4 depicts UV spectral wavelength scan after affinity chromatography of recombinant mature Con A (refolded from the fraction insoluble in MOPS-metals buffer obtained from glycogen-deficient mutant bacterial cells);

FIGURE 5 depicts dextran affinity chromatography elution profile for recombinant mature Con A refolded after using the Borate Wash Method;

FIGURE 6 depicts UV spectral wavelength scan following affinity chromatography of recombinant mature Con A refolded after using the Borate Wash Method;

FIGURE 7 depicts stained SDS-PAGE showing repeated purification experiments on recombinant mature Con A using the Borate Wash Method;

FIGURE 8 depicts MALDI-TOFF mass spectrogram following affinity chromatography of recombinant mature Con A refolded after using the Borate Wash Method;

FIGURE 9 depicts dextran affinity chromatography elution profiles for recombinant mature Con A: performance of the Borate Wash Method on (A) cells grown in medium without added glucose and (B) glycogen-replete cells;

FIGURE 10 depicts UV spectral wavelength scans following affinity chromatography of recombinant mature Con A: performance of the Borate Wash Method on (A) cells grown in medium without added glucose and (B) glycogen-replete cells;

FIGURE 11 depicts phenol-sulphuric acid analysis for non-dialysable carbohydrate present in a recombinant mature Con A preparation from glycogen-replete cells made using the Borate Wash Method;

FIGURE 12 depicts demonstration by iodine color reaction of the presence of glycogen in precipitated material formed during the refolding of recombinant mature Con A after performing the Borate Wash Method on cultures grown with or without added glucose; and

FIGURE 13 depicts scheme of preferred embodiments of method for example of recombinant forms of Concanavalin A.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE PRESENT INVENTION